

# BIOLOGICAL BULLETIN

## SELECTION FOR HIGHER AND LOWER FACET NUMBERS IN THE BAR-EYED RACE OF *DROSOPHILA* AND THE APPEARANCE OF REVERSE MUTATIONS.<sup>1</sup>

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### THE EXPERIMENTS OF ZELENY AND MATTOON.

In 1915 Charles Zeleny and E. W. Mattoon published their results obtained by selecting for higher and lower facet numbers in the bar-eyed race of *Drosophila*. Three lines were selected for higher numbers and three lines for lower numbers. Individual pairs were mated and the selection was carried on for three generations in each direction.

They counted the facets in 250 flies at the beginning of the experiment and found the average number to be 98.04 for males and 65.06 for females. Counts made on 250 more flies from the stock at the end of the experiment gave the same results. From the ratio between numbers from males and females they computed a factor for converting the female number to the male number and published all numbers on the male standard. Without exception each generation produced successively higher

<sup>1</sup> Contribution from the Zoölogical Laboratory of the University of Illinois, No. 97.

numbers in the upward selected lines and successively lower numbers in the downward selected lines, and in each case the three lines were very close together. The average facet number was raised from 98.0 to 139.5 and lowered from 98.0 to 83.7. The maximum was raised from 182 to 213 and the minimum was lowered from 45 to 36. This increase in the range was not due to a larger number of counts, as 500 flies were counted from the stock and only 450 from the selected lines.

#### THE EXPERIMENTS OF MACDOWELL.

In the same year E. Carlton MacDowell published the results of selections for higher bristle numbers in a race of *Drosophila* with extra bristles on the thorax. For six generations the number of extra bristles increased, but failed to rise any higher in forty additional generations. The response to selection was not so definite as in the experiments of Zeleny and Mattoon. The variation in the averages from different lines was very large. While the average of a large number of lines increased with each generation, that of any particular line often decreased for a generation. This wide range of fluctuations in the averages from different lines was partly explained by the existence of a correlation between the number of extra bristles and the size of the fly.

There is, however, another possible cause that was apparently overlooked. MacDowell selected for extra bristles in two arbitrarily limited rows on the dorsal side of the thorax, but states that even when no extra bristles were present in those rows "these flies frequently showed extra bristles on other parts of the thorax." The factors for extra bristles evidently controlled not only the number of extra bristles in those rows, but also the number of extra bristles on other parts of the thorax and possibly over the entire body. Selection, then, was made for only a small part of a variable character. A high number of bristles in the area under observation would in general indicate a high number of bristles on other parts of the body, but might actually be accompanied by a low number of extras elsewhere. Low offspring from high parents could be accounted for in that way. The efficiency of the selection would also

decrease as the part of the character under observation decreased in comparison with the part not under observation.

#### THE PRESENT EXPERIMENTS.

Since the experiments of Zeleny and Mattoon gave such clear results, but were interrupted before they led to a final conclusion, it seemed desirable to repeat them on a larger scale and continue them for a greater number of generations to determine if a pure line could be established and to study the changes in the existing factor or factor complex.

#### MATERIAL AND METHODS.

In order to have a check on any possible contamination a stock was selected which had a second recessive character, the vestigial wing, in addition to the bar eye. This stock also had the advantage of having on the average a lower facet number than the long-winged stock. But sterility in the race and the indefinite character of the results made it necessary, after a few generations, to return to the long-winged stock. In both cases the eye color was that of the normal wild fly.

The vestigial-winged, bar-eyed stock was designated as VBa; the long-winged, bar-eyed stock as Ba. The downward and upward selected lines in VBa were distinguished by l and h, in Ba by d and u respectively. Individual lines were distinguished by numbers. Any given mating received the number of the generation to which it belonged and a serial number corresponding to the number of matings made from that line in the given generation. In that way a number like Bau4f2-5, although cumbersome, gives nearly the whole pedigree of the mating involved. Since the lines are now clearly distinguished without the stock designations, Ba and VBa, those designations will frequently be omitted in this paper.

The material for the present set of experiments was obtained from Professor Charles Zeleny on whose advice the work was undertaken. To him the author is also indebted for a keen interest in the work and for many helpful suggestions.

All stocks were kept in large bottles while eight dram vials were used for individual pairs. The vials were plugged with





became hard, and larvæ did not seem to thrive well in it. This defect, however, was remedied after one or two generations had been reared from the vestigial-winged stock and long before the experiments on the long-winged stock were undertaken.

In some preliminary work the number of facets was estimated in the eyes of the parents selected and the actual counts were made at the time the parents were killed. This method was employed by Zeleny and Mattoon. The author, however, found that his estimates were not close enough and that in some cases parents were lost because they died and were destroyed by the larvæ before their death was discovered. The facets of dead flies also disintegrate when they remain in contact with food for a short time. For those reasons all selections during the experiments were made from actual counts.

During all of the work on the vestigial-winged flies and during part of the work on the long-winged flies, selections were made at noon and night after all the flies had been removed in the morning. In that way the specimens were never more than six hours old at the time of selection. But observations during that time showed that males seldom or never mated before they were twenty-four hours old, and for that reason later selections were made every twelve hours. All desirable specimens were saved and when no mates were present they were kept in vials with food until matings could be made.

All flies were etherized and the facets were counted while they were quiescent. The larvæ were never subjected to ether, as the flies to be examined were first transferred to empty vials. Selected flies recovered from the effect of the ether in fifteen minutes to half an hour. The other flies were preserved in 85 per cent alcohol.

For counting facets the flies were placed in a little pit on a paraffin block and illuminated by means of a 25-watt tungsten lamp. This gave sufficient light but only a moderate amount of heat. A Leitz microscope with a number four ocular and a number three objective and the tube drawn out to its full length was found to be most convenient. This gave a relatively high magnification with a suitable depth and size of field.

Errors in counting can not be avoided; it is merely a question

of reducing them to a minimum. In eyes with 100 facets or less the error is certainly less than one per cent., but it increases rapidly as the facet number increases. Recounts made on eyes

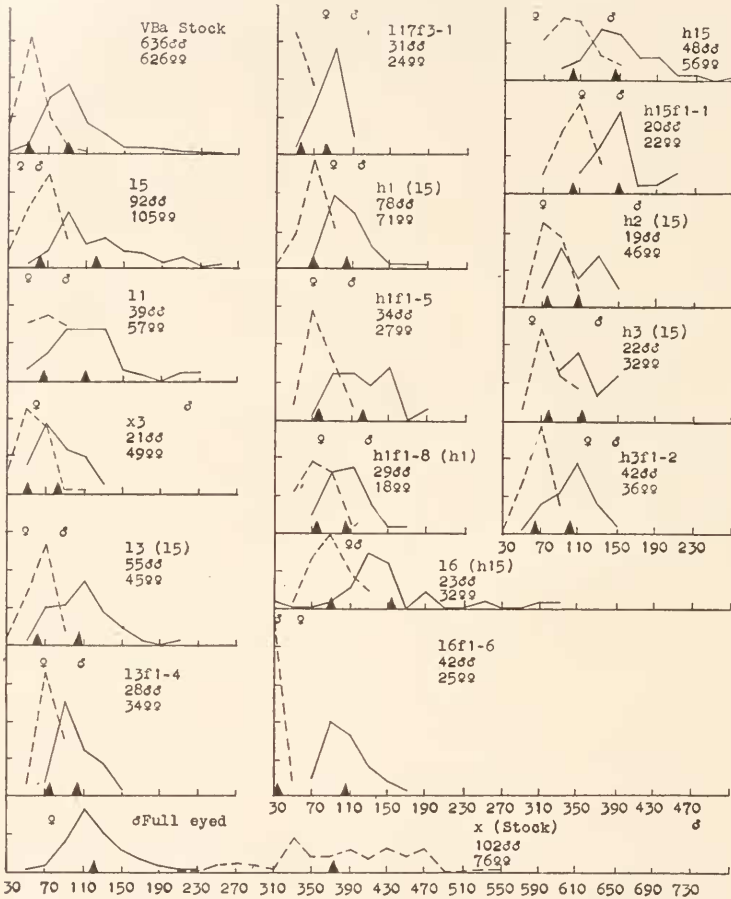


FIG. 2. Curves showing the effect of selection in individual lines in VBa. All curves are plotted on the scale of fifty. The males are represented by continuous lines and the females by interrupted lines. The parents are indicated at the top and the averages by the black pyramids below. The values plotted are for flies between 20 and 39, 40 and 59, 60 and 79, etc. The number of the mating is given above, followed by the source in brackets unless the parents come from the mating represented by the curve just above. In case of difficulty in determining the relationship of the lines consult Fig. 1.

with as many as 300 facets showed an error of possibly two or three per cent. Of course it takes two or three weeks of practice to reach that point of efficiency.

Errors are due chiefly to the following causes: (1) The arrangement of the facets may be irregular. Ordinarily the facets are arranged in rows and one can easily count two rows at a time even if the eye is large. But in the bar eye the dorsal part usually contains a large number of facets without any indication of rows. This area increases very rapidly with the size of the eye. In heterozygous females the facets are all arranged in beautiful rows, and one is thus able to distinguish readily between the eye of a heterozygous female and a bar-eyed male even if both eyes contain the same number of facets. Bar-eyed females as a rule contain more regular facets than the males and the distinction between low heterozygous females and high bar-eyed females is more difficult. (2) There may be small facets either among the others or at the margins. These facets are most abundant in irregular eyes and may be one of the causes of irregularity. All grades are found from mere prominences that can only be seen with the most advantageous light to normalized facets. As a rule, however, there are few if any doubtful facets. (3) There may be colorless facets at the margins. Normally the colored area of the eye extends somewhat beyond the facet area, but in some rare cases a few facets may extend beyond it and these may be overlooked. (4) When the eye is so large that the fly must be turned to count all of the facets some of the facets in the middle may be recounted or omitted. All heterozygous females and some high grade males had to be turned. The error in this case also is not very great, as the bar eye as well as the heterozygous eye is almost divided in the middle. (5) Errors may also arise through a lack of concentration or through the inability to make the eye retain its position. These are purely personal elements.

No mechanical device was used for marking off the counted area from the uncounted area on the eye of the fly. The author relied entirely on the ability of his eye to follow the rows or, in the absence of rows, to mark off certain areas and hold them until the eye was counted. A cross-hair in the ocular was tried, but was found to be unreliable. It could be used if the eye of the fly presented a flat field, but with the rounded contour of the eye the hair keeps on traveling over facets as one focuses up

and down and comes to rest on the same facets only in the same focal plane. But it is impossible to count all facets without large changes in the focus. In shifting the fly after a given area has been counted it is evident that the same shift makes the hair pass over more facets on a slanting area than on a horizontal one, just as surveyors will pass over more surface in surveying up or down a hill than on the level. But on the rounded eye the hair usually passes over both a horizontal and a slanting area at the same time. For that reason one can not possibly shift so that the hair crosses the same number of facets at every point on the eye. A cross-ruled ocular has little or no advantage over a cross-hair, and a camera lucida is scarcely worthy of consideration as it is difficult enough to keep it at the proper place even on a perfectly flat field.

The largest error, by far, is due to the fact that only the right eye was counted. Zeleny and Mattoon reported that they found the averages of a large number of counts to be the same for both eyes. The author obtained the same results. But that does not mean that both eyes in any given fly have the same number. Normally the variation is not more than about 1 per cent from the mean, but the author has found it as high as 5 per cent, and in one case 10 per cent. One abnormal male was obtained with 25 facets in the right eye and 146 in the left. It is obvious that the error is greatest in the parents and the extremes of the offspring as we are dealing in those cases with individual flies. In parents the left eye was usually examined to see that it was not abnormal, and actual counts were made in a large number of cases. The abnormal male was mated to see if the unequal condition would be inherited, but it died within twenty-four hours without giving any offspring.

#### SELECTIONS IN THE VESTIGIAL-WINGED STOCK.

When the bottle that was to give rise to the VBa stock was received, flies were transferred to two fresh stock bottles and the offspring from these parents were used for making the original selections. From October 31 to November 5 some preliminary counts were made on flies that hatched in the original bottle. Sixty-nine males gave a mean facet number of 115 and seventy females gave a mean of 63.

All of the offspring from the parents placed into the new stock bottles were counted, including the ones that were used to continue the stock, and selections were made during the entire period. From the preliminary counts it was concluded that flies above a certain limit could be selected as high and those below another limit as low; but when the selections were begun it was found that no flies appeared that approached the lower limit set for high selections and many flies were below the limit set for low selections. As a result a new standard had to be established. Later the stock became more variable and some higher flies appeared together with some very low ones; indeed the lowest female obtained during these experiments was taken from one of these bottles. In all 370 males gave an average of 96 facets and 364 females gave an average of 54 facets. More counts on the stock were made March 17 to April 2, and this time 197 males gave an average of 82 facets and 192 females, an average of 44. This variability of the stock was contrary to the conditions found by Zeleny and Mattoon and seriously interfered with the success of the present experiments. It was necessary to pay as much attention to the nature and possible cause of the variation as to the effect of selection.

The greatest difficulty encountered in the VBa selections, however, was sterility and low production in the single lines. Of 42 matings made from the stock only 6 were fertile and of these only 2 gave a sufficient number of offspring for selection. On the whole about 80 per cent of the matings were sterile, and in many cases even lot matings failed to produce any offspring. By the end of the fifth generation all lines had died out except h17 and h15, and these were saved only through lots. For that reason the experiment was discontinued at that point.

No special experiments were made to determine the cause of sterility. Obviously it was not due to inbreeding, for it was as great in the first generation as in succeeding ones. In the majority of cases it was also not due to the inability of the females to deposit eggs as was the case in the experiments of Hyde. A few of such abnormal females were observed, but in most cases eggs appeared in the food and failed to hatch. The

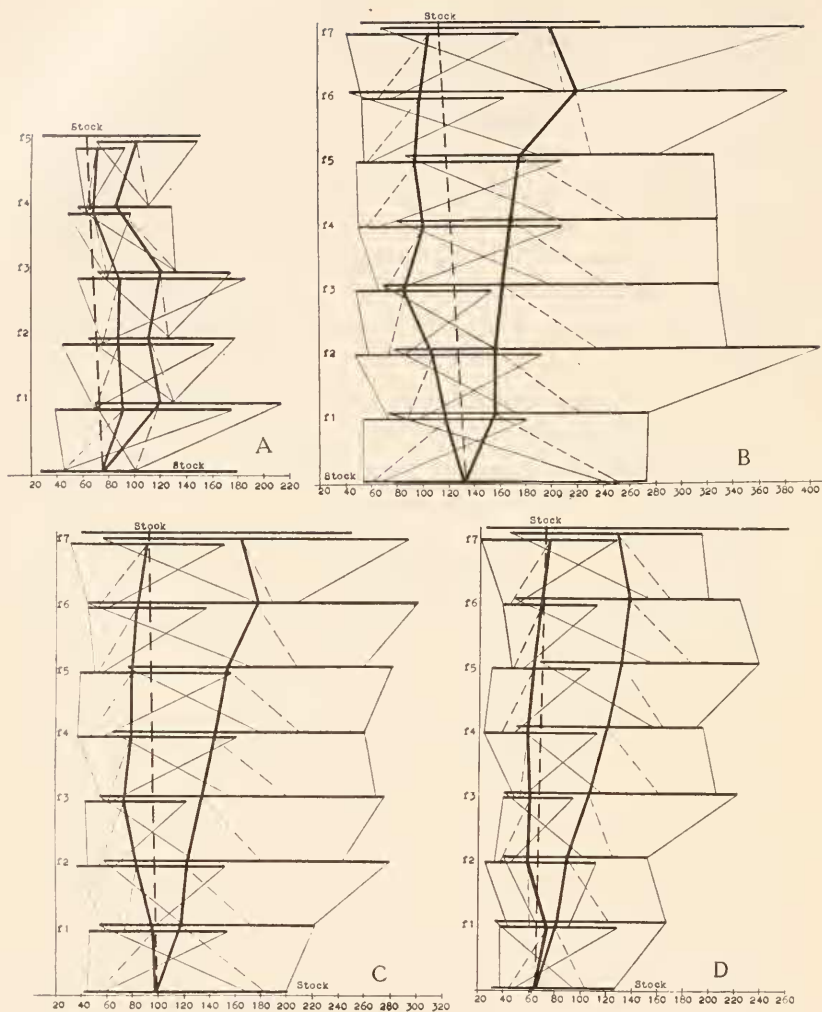


FIG. 3. The effect of selection on range and average. A, high and low lines in VBa; B, males in Ba, high and low lines; C, high and low lines in Ba; D, females in Ba, high and low lines. A and C are plotted for the means between male and female averages. These figures do not represent individual lines, but include all lines except reverse selections for the generations indicated. The heavy horizontal lines indicate the range, the heavy vertical lines, the averages. The heavy interrupted vertical lines merely connect the initial and final stock averages and do not indicate the averages of the stock at intermediate points. The fine slanting lines connect the extremes of the parents with those of the offspring while the fine interrupted lines connect the averages of the parents with those of the offspring. The facet numbers are indicated at the bottom, the generations at the left hand side.



results of Castle correspond more nearly to the facts observed.

The results obtained from these selections can best be made out by consulting Table I. and Figs. 1, 2 and 3.

The attempt to select downward was entirely unsuccessful. The selection was never very rigid, but the average of the offspring persistently remained above that of the stock.

The upward selections were more successful. In h15 the males rose from the stock average of 96 to 147 and the females from 54 to 101. In the other two lines where smaller numbers of offspring were obtained the rise was not quite so large. The second and third generations, however, failed to give any further increase in the facet number. Upward selections taken from line 15 after the first generation failed to give any rise in the facet number. The line x3 was taken from 11 after two generations of downward selection and gave a decrease in the facet number in spite of the fact that the male had 222 facets and was mated to average females.

The line 16 was intended to be a downward selection from h15, but the parents proved to be very near the mean and all matings from the first generation were sterile except f1-6. This particular mating involved a male with 34 facets, the lowest number obtained in these experiments. Fortunately a fair number of offspring was obtained. The males average much lower than those of the previous generation and about the same as those of the low lines hatching at the same time, but the females average much lower than anything obtained elsewhere. It is likely that the male with 34 facets was a mutant and that the mutation chiefly concerned the sex chromosome. The complete sterility of this race prevented any further investigation.

The results here obtained are very different from those reported by Zeleny and Mattoon. In their selections all three generations went gradually upward and gradually downward; in these experiments there was a sudden rise in the upward selected lines in the first generation and no further effect, and no response at all in the downward selected lines. For that reason and also on account of the high degree of sterility it seemed best to make some selections in the long-winged stock used by Zeleny and Mattoon.



TABLE I.

RELATION OF PARENTS AND OFFSPRING. VESTIGIAL-WINGED, BAR-EYED STOCK (VBa).

Parents.				Offspring.							
Bottle Number.	Source.	Facets.		♂				♀			
		♂	♀	No. Flies.	Facets.			No. Flies.	Facets.		
					Ave.	Max.	Min.		Ave.	Max.	Min.
Stock	Stock			636	93.8	253	36	626	51.9	108	19
l4	Stock	63	46	3	101.7	134	85	12	71.9	93	38
l5	Stock	60	45	92	120.2	254	56	105	63.2	98	23
l1	l5	86	48	39	110.3	222	57	46	67.4	95	41
l1f2	l1	64	46	10	116.5	269	77	12	73.8	104	56
l2	l5	56	37	13	107.3	142	70	14	69.2	88	52
l3	l5	98	53	55	106.3	208	56	45	63.6	88	32
l3f1-4	l3	108	71	28	102.9	149	76	34	74.7	97	51
l3f1-10	l3	96	53	9	116.1	142	82	10	77.6	96	56
l3f1-16	l3	75	56	1	121.0			1	86.0		
l3f2-3	l3f1-4	78	51	2	57.0	58	56	5	50.2	54	47
l17	Stock	46	19	2	98.0	108	88	2	85.5	90	81
l17f2	l17	108	90	4	118.8	122	116	8	92.4	102	83
l17f2-3	l17f2	116	83	12	100.9	165	70	17	60.1	89	42
l17f3-1	l17f2-3	113	81	31	85.0	117	57	24	56.3	76	41
l17f4-3	l17f3-1	77	54	9	84.2	96	70	3	64.0	74	51
l17f4-5	l17f3-1	lot	lot	16	82.4	108	60	11	57.2	77	48
l17f4-12	l17f3-1	lot	lot	2	90.0	100	80	6	59.8	65	53
h1	l5	120	86	78	106.2	182	71	92	70.1	99	29
h1f1-5	h1	108	74	34	122.0	192	78	27	77.7	101	58
h1f1-8	h1	128	75	29	107.3	160	75	18	73.6	103	52
h2	l5	170	73	19	110.7	163	68	46	79.4	105	53
h2f2	h2	156	90	5	146.2	175	125	6	89.3	113	79
h2f1-5	h2	163	100	4	88.0	112	72	6	58.7	69	48
h2f2-1	h2f2	159	113	2	103.5	115	92	6	59.7	70	50
h2f3-1	h2f2-1	115	70	14	109.6	143	88	13	67.4	89	55
h3	l5	126	64	22	114.8	154	82	32	80.0	116	57
h3f2	h3	145	116	42	102.7	150	52	36	67.4	95	29
h3f2-2	h3f2	122	90	3	80.3	85	72	1	65.0		
h14	Stock	155	64	9	128.3	190	95	3	89.0	108	77
h14f2	h14	161	108	4	94.0	113	74	9	69.0	82	63
h15	Stock	137	60	48	147.1	271	81	56	101.2	158	70
h15f1-5	h15	123	96	17	121.7	154	84	14	83.6	113	56
h15f2	h15	148	112	20	149.7	219	105	22	102.5	137	64
h15f2-3	h15f2	lot	lot	16	147.2	199	92	19	91.4	125	51
h15f2-5	h15f2	205	111	16	151.6	217	118	8	94.0	129	57
h15f3-1	h15f2-3	156	109	10	96.7	126	76	8	73.5	123	59

Parents.		Offspring.									
Bottle Number.	Source.	Facets.		♂				♀			
		♂	♀	No. Flies.	Facets.			No. Flies.	Facets.		
					Ave.	Max.	Min.		Ave.	Max.	Min.
h15f3-3	h15f2-3	150	107	3	84.0	97	62	3	76.7	85	61
h15f3-6	h15f2-3	158	102	9	97.0	134	63	19	73.8	103	50
h15f4-4	h15f3-6	131	103	7	120.6	196	79	4	89.3	100	65
h15f4-9	h15f3-6	lot	lot	3	117.3	144	88	3	78.7	87	63
h15f4-11	h15f3-6	134	93	2	124.0	131	117	3	74.3	86	63
h18	Stock	163	62	12	123.8	241	90	11	94.8	129	59
16	h15	122	108	23	151.3	338	34	32	89.6	127	56
16f1-6	16	34	64	42	105.1	170	70	25	33.5	56	25
x	Stock	full	43	102	122.3	228	56	76	377.9	543	218
x3	11	222	lot	21	82.0	127	56	49	54.9	104	31
x4	16	328	lot	12	142.0	243	70	16	77.4	116	53
x4f1-1	x4	140	116	5	146.0	181	130	10	111.5	135	83
x4f2-5	x4f1-1	132	103	6	169.5	196	143	1	119.0		

## SELECTIONS IN THE LONG-WINGED STOCK.

These experiments were begun on January 15 and continued until the middle of June. In this case the number of flies counted from the stock during selection was 91 males with an average facet number of 132 and 81 females with an average of 66. The first selection made was very near the mean and was designated as Ba1 and used as part of the stock. It yielded 44 males and 37 females with mean facet numbers of 125 and 68 respectively. In June the eyes of 73 additional males and 97 additional females from the stock were counted, yielding mean facet numbers of 114 and 72 respectively. Here again, as in the vestigial-winged stock, there is a change in the mean facet number, and in this case it also involves a decided change in the ratio between male and female facet numbers.

Little difficulty was encountered on account of sterility in this stock. About 75 per cent of the matings were fertile and nearly 75 per cent of the fertile matings produced 50 offspring or more. In each case all of the offspring were counted. To avoid the dying out of lines a number of branches of each line was run, only brothers and sisters were mated, but no attention was paid to the dying out of any given branch when it did not

come up to the standard in facet number or production. In that way the most suitable flies from the most suitable branches were selected, bringing the selections to some extent on the behavior basis. By that method parents whose somatic constitution varies from their germinal constitution are to some extent eliminated.

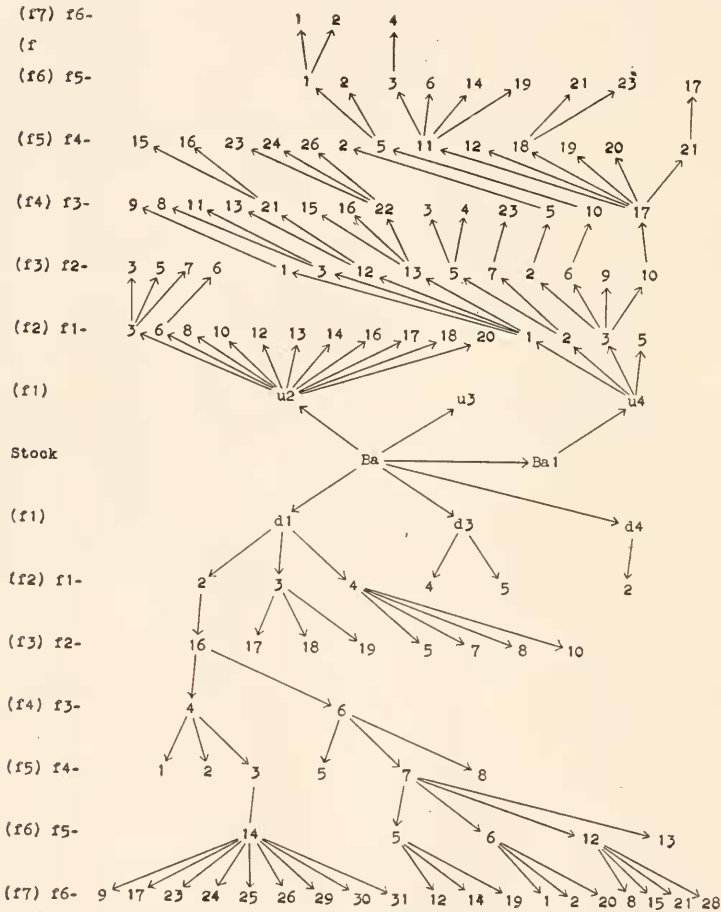


FIG. 4. Relation of selected lines and stock in Ba.

Selections in this stock were carried on for seven generations in each direction, several return selections were made, and crosses in both directions were finally made from the selected lines. The results are shown in Table II. and in Figs. 3, 4, 5, 6 and 7.

The downward selections were not very effective. In the first generation the average facet number in the females actually rose above that of the stock, but the average of the males dropped

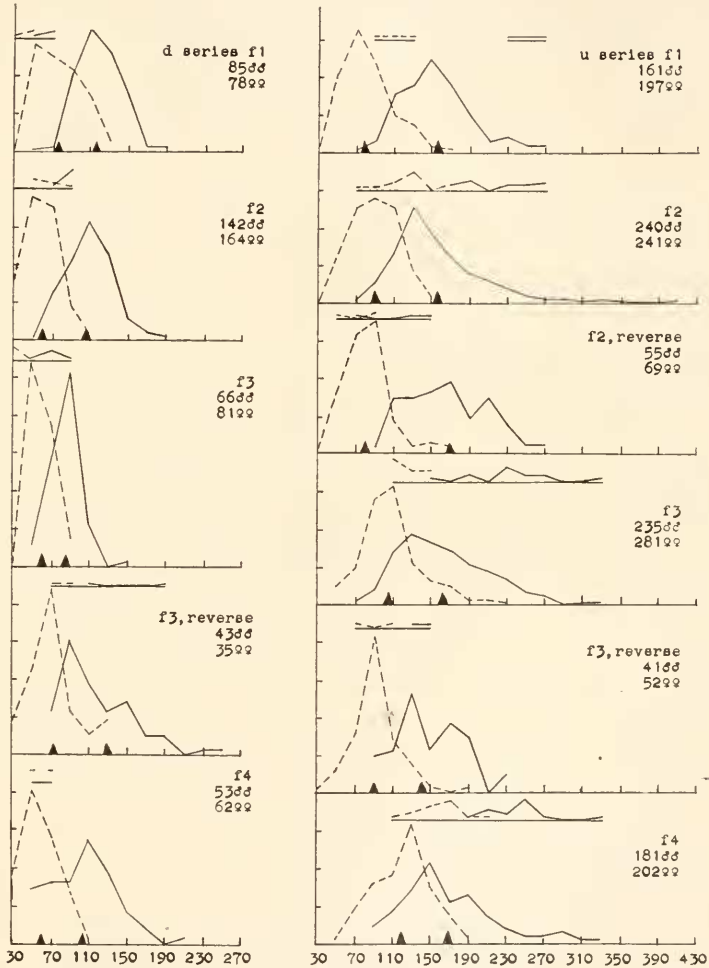


FIG. 5A.

considerably below. In the next generation the females dropped decidedly and the males dropped only slightly. The third generation produced another slight lowering of the means, and after that there was a gradual return toward the mean of the stock.

In the seventh generation the mean of the stock had been reached by the females, but the males were still slightly lower. Taking the mean of the male and female averages more uniform results appear to have been obtained. In that case the first generation shows no change, the second and third generations show a gradual

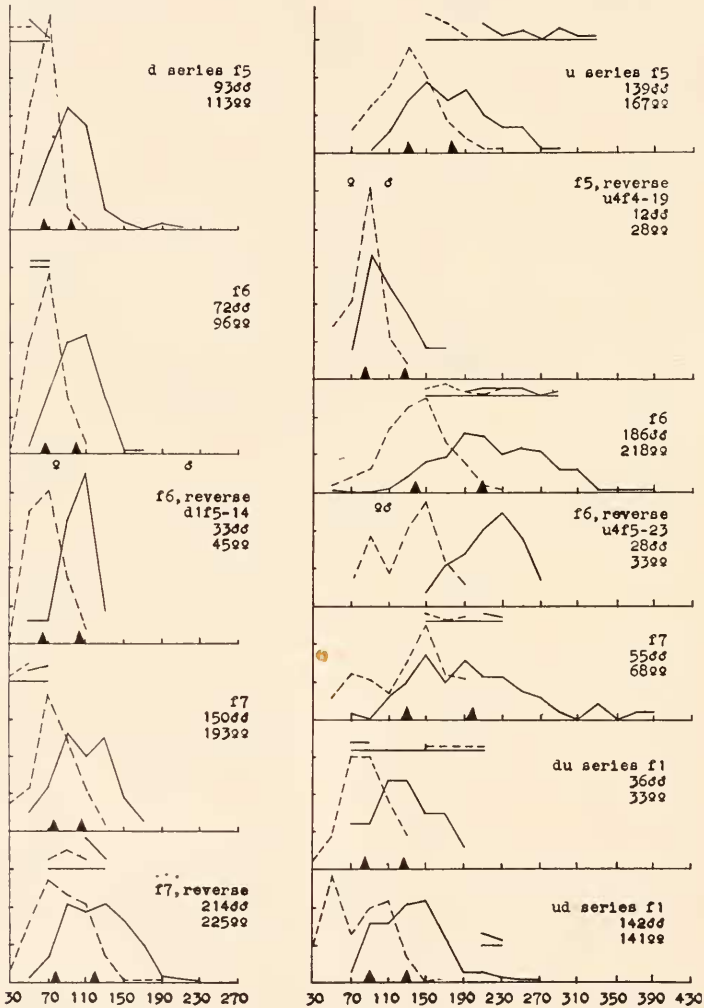


FIG. 5B.

FIG. 5. Curves showing the effect of selection for generations in Ba. These curves are plotted on a scale of one hundred. Parents are indicated by smaller curves at the top. In other respects the curves are like those of Fig. 2.

lowering, but the next four generations show a complete return to the mean of the stock.

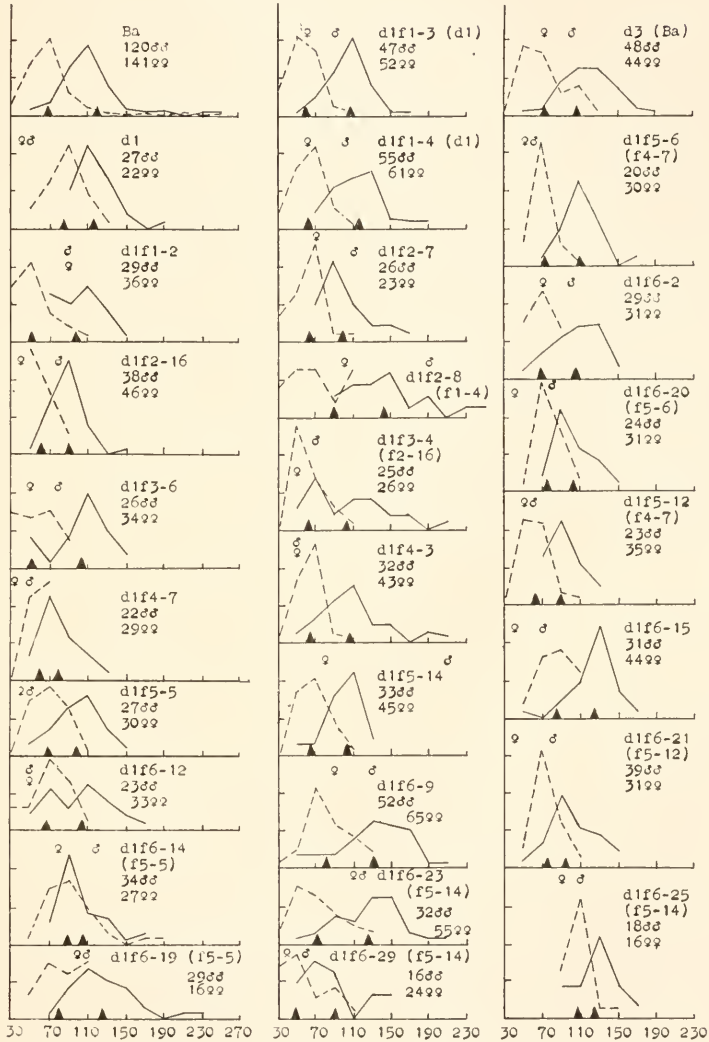


FIG. 6. Curves showing the effect of selection in individual lines in Ba, downward selections. For other information consult Fig. 2.

The upward selections were more successful. With the exception of the last generation there is in every case a slight rise

in both sexes. The results in the last generation can be explained by the fact that the selections were below the average

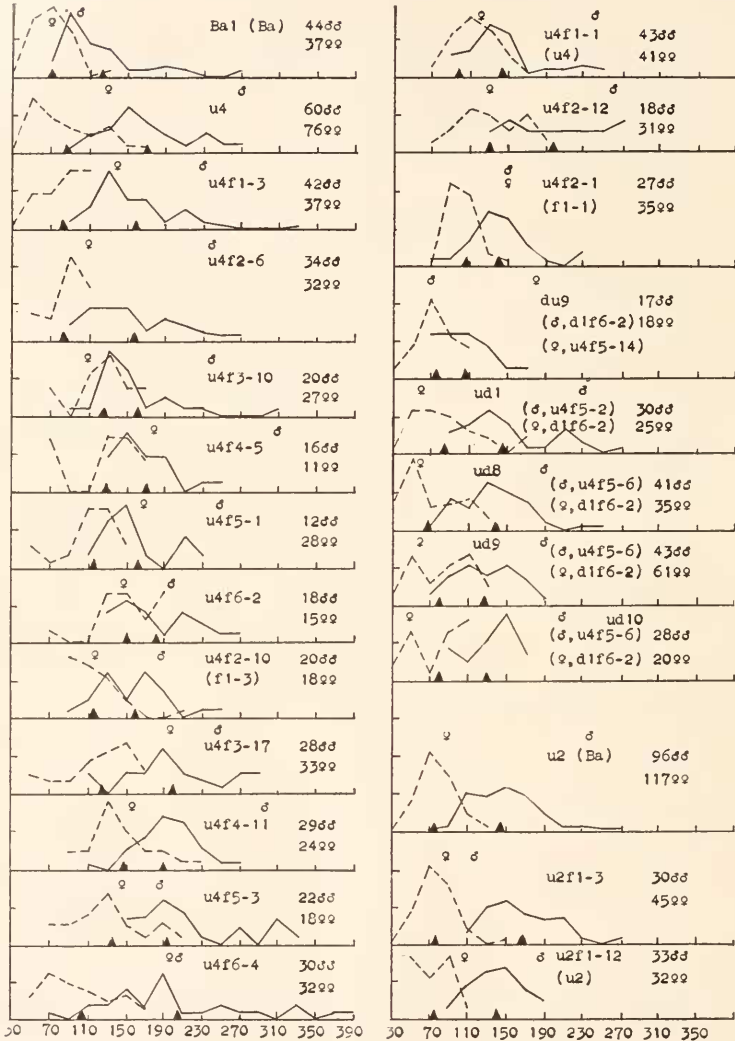


FIG. 7. Curves showing the effect of selection in individual lines in Ba, upward selections and crosses. For other information consult the description of Fig. 2.

in the males and not far above in the females, and the fact that the offspring hatched at a time when all counts were low. This will be explained later.



TABLE II.

RELATION OF PARENTS AND OFFSPRING. LONG-WINGED, BAR-EYED STOCK (Ba).

Parents.				Offspring.								
Bottle Number.	Source.	Facets.		♂					♀			
		♂	♀	No. Flies.	Facets.			No. Flies.	Facets.			
					Ave.	Max.	Min.		Ave.	Max.	Min.	
Ba	Stock			120	122.7	240	53	141	69.1	259	24	
Ba1	Stock	104	74	44	125.0	273	69	37	68.3	128	34	
d1	Stock	53	38	27	117.4	180	91	22	84.0	122	56	
d1f1-2	d1	92	91	29	99.5	141	64	36	52.5	102	25	
3	d1	92	56	47	106.0	164	58	52	58.4	108	28	
4	d1	98	65	55	114.7	192	62	61	59.4	111	32	
d1f2-5	d1f1-4	84	62	0				1	65.0			
7	4	106	73	26	97.9	162	62	23	62.6	117	37	
8	4	190	98	17	148.6	242	85	12	90.0	131	47	
10	4	87	47	11	87.1	104	67	11	63.4	87	48	
16	2	76	36	38	87.6	153	58	46	61.6	92	40	
17	3	75	36	12	72.3	95	49	17	54.5	71	38	
18	3	68	35	4	80.0	87	73	6	63.7	73	58	
19	3	58	33	1	109.0			1	64.0			
d1f3-1	d1f2-10	76	48	2	88.5	90	87	2	50.0	53	47	
4	16	68	54	25	103.2	209	51	26	60.6	111	26	
6	16	79	53	26	101.8	154	57	34	55.1	98	23	
d1f4-1	d1f3-4	57	48	11	98.9	122	74	7	64.6	68	59	
2	4	51	44	11	90.2	107	79	13	70.2	88	49	
3	4	55	47	32	109.4	208	55	43	62.2	104	37	
5	6	59	32	4	87.0	96	75	6	66.8	82	37	
7	6	57	23	22	80.3	133	56	29	59.1	77	29	
8	6	69	35	13	91.2	115	50	15	63.5	91	49	
d1f5-5	d1f4-7	56	45	27	98.9	155	54	30	70.8	100	37	
6	7	61	46	20	111.5	165	78	30	72.3	105	45	
12	7	56	55	23	90.2	127	65	35	61.3	110	39	
13	7	62	51	2	78.5	83	74	1	71.0			
14	3	208	77	33	101.1	132	56	45	66.6	116	30	
d1f6-1	d1f5-6	165	105					1	67.0			
2	6	102	70	29	108.2	155	59	31	69.7	99	42	
8	12	126	110	2	102.5	103	102	3	85.0	90	81	
9	14	125	93	52	131.5	215	50	65	82.0	139	27	
12	5	54	49	23	103.7	166	42	33	69.3	104	22	
14	5	122	83	34	103.3	164	69	27	93.8	187	52	
15	12	68	45	31	123.4	167	51	44	86.1	114	44	
17	14	119	92	1	121.0							
19	5	111	100	29	127.3	237	71	16	84.6	117	52	
20	6	78	45	24	101.9	144	72	31	78.9	112	49	
21	12	78	39	39	97.4	153	51	31	72.7	104	40	
23	14	116	110	32	125.3	206	51	55	70.9	135	23	

TABLE II.—Continued.

Bottle Number.	Parents.		Offspring.								
	Source.	Facets.		♂				♀			
		♂	♀	No. Flies.	Facets.			No. Flies.	Facets.		
					Ave.	Max.	Min.		Ave.	Max.	Min.
d1f6-24	14	114	94	13	135.5	185	81	16	104.2	127	73
25	14	113	90	18	125.1	163	89	16	107.5	145	86
26	14	108	66	20	105.0	147	51	7	87.3	121	49
28	12	77	48	14	127.1	176	72	24	88.0	125	30
29	14	57	43	16	90.8	158	56	24	53.9	109	20
30	14	117	72	13	115.5	178	78	19	65.8	105	42
31	14	56	30	3	88.3	95	83	6	71.2	90	46
d3	Stock	65	51	48	118.2	181	54	44	72.8	128	37
d3f1-4	d3	74	43	1	92.0			7	72.0	92	54
5	d3	84	45	2	59.0	70	48	1	45.0		
d4	Bar	69	48	10	116.3	145	81	12	66.9	86	50
d4f1-2	d4	90	61	8	128.6	157	108	5	64.2	78	54
u2	Stock	238	94	96	147.6	268	74	117	77.5	132	39
u2f1-3	u2	122	89	30	105.7	261	103	45	75.2	141	32
6	u2	144	94	15	187.7	249	107	14	90.9	162	52
8	u2	172	92	28	172.1	408	79	26	90.2	148	59
10	u2	187	132	22	191.8	330	125	14	104.1	144	69
12	u2	190	111	33	141.6	191	86	32	74.7	113	43
13	u2	192	109	16	144.6	203	90	18	94.4	135	49
14	u2	220	110	2	175.5	186	165	4	116.5	130	105
16	u2	246	123	8	126.3	153	106	10	95.1	111	75
17	u2	268	126	5	206.6	278	130	4	116.3	124	110
18	u2	172	63	22	141.7	249	78	38	81.0	138	49
20	u2	74	56	10	127.1	221	90	10	90.5	100	77
u2f2-3	u2f1-3	190	102	15	157.0	240	88	16	91.3	116	72
5	3	261	146	1	136.0			2	121.5	134	109
6	6	220	114	4	114.0	120	104	2	81.0	88	74
7	3	136	76	6	107.7	138	81	6	77.0	88	68
u3	Stock	240	109	5	124.8	138	98	4	101.8	120	93
u4	Bar	273	128	60	170.9	275	99	76	85.3	167	34
u4f1-1	u4	241	121	43	146.3	255	83	41	94.5	151	49
2	u4	271	160	18	154.2	274	100	15	105.5	140	66
3	u4	227	136	42	158.2	336	96	37	83.1	116	39
5	u4	275	167	1	161.0			2	100.5	118	83
u4f2-1	u4f1-1	153	151	27	141.4	231	74	35	106.0	173	61
2	3	147	106	35	153.3	221	93	46	92.1	191	35
3	1	225	116	51	151.6	331	70	41	87.3	144	40
5	2	223	140	13	179.4	224	112	11	110.0	173	51

TABLE II.—Continued.

Parents.		Offspring.											
Bottle Number.	Source.	Facets.		♂						♀			
		♂	♀	No. Flies.	Facets.			No. Flies.	Facets.				
					Ave.	Max.	Min.		Ave.	Max.	Min.		
u4f2-6	3	237	111	34	156.9	263	83	32	86.4	113	40		
7	2	274	129	31	166.1	238	107	54	109.9	217	45		
9	3	336	100	8	179.1	229	150	11	108.3	169	85		
10	3	187	118	20	156.8	244	99	18	114.8	205	81		
12	1	255	127	18	199.1	266	126	31	131.0	190	66		
13	1	252	122	13	203.4	267	119	28	126.3	221	69		
u4f3-3	u4f2-5	215	173	3	135.7	165	99	6	147.0	193	84		
4	5	213	147	7	160.4	182	125	14	124.9	166	61		
5	2	197	121	30	173.1	330	92	23	112.1	154	75		
8	3	331	173	7	142.9	198	108	2	151.5	166	137		
9	1	231	143	3	148.0	190	117	6	124.7	158	113		
10	6	241	112	20	159.2	308	99	27	126.5	170	64		
11	3	242	136	8	156.5	207	129	7	115.3	127	96		
13	12	260	190	12	194.7	291	140	14	126.2	150	89		
15	13	245	172	4	149.5	185	113	14	114.6	148	83		
16	13	221	172	9	139.3	167	112	6	102.0	136	70		
17	10	244	205	28	201.0	298	108	33	122.2	168	48		
21	12	255	179	18	175.5	294	132	16	122.0	167	65		
22	13	255	145	15	178.0	244	99	25	115.6	190	67		
23	7	216	145	17	127.1	281	82	9	94.3	149	75		
u4f4-2	u4f3-5	330	154	22	159.3	260	88	20	99.7	141	67		
5	10	308	176	16	169.9	240	129	11	125.6	173	69		
11	17	290	157	29	191.6	269	101	24	145.3	236	81		
12	17	298	158	5	144.4	168	120	4	129.5	141	125		
15	21	203	156	2	165.5	167	164	7	114.0	137	77		
16	21	220	165	4	155.0	187	138	4	138.5	158	129		
18	17	284	156	15	190.6	284	106	27	134.2	237	77		
19	17	109	67	12	128.9	192	77	28	84.1	127	52		
20	17	250	155	3	153.7	196	109	6	120.2	141	86		
21	17	209	152	11	186.0	256	132	8	141.1	195	84		
23	22	214	163	13	173.7	257	125	17	123.2	160	78		
24	22	244	190	17	186.8	327	130	37	136.1	182	85		
26	22	209	161	2	120.0	121	119	2	131.5	135	128		
u4f5-1	u4f4-5	238	173	12	158.3	222	111	28	113.3	153	46		
2	5	240	167	14	182.2	235	118	10	135.3	162	76		
3	11	189	153	22	195.6	302	123	18	133.9	209	76		
6	11	213	151	42	225.2	384	45	37	127.6	177	65		
14	11	249	182	43	247.0	367	143	50	137.7	198	72		
17	21	205	162	31	242.2	354	151	28	148.5	201	58		
19	11	231	236	14	215.7	303	140	40	151.0	221	92		
21	18	284	237	8	192.5	210	170	7	151.3	185	103		
23	18	106	98	28	217.2	278	151	33	130.4	186	64		
u4f6-1	u4f5-1	222	153	7	213.3	260	116	21	152.2	191	81		
2	1	201	149	18	184.1	262	128	15	149.3	191	76		
4	3	209	196	30	204.4	398	69	32	99.1	174	43		



TABLE IV.

DAILY COUNTS, CROSSES BETWEEN HIGH MALES AND LOW FEMALES.  
(Males from u4, Females from dl.)

Date.	Baudr.				Baud8.				Baud9.				Baudro.	
	♂		♀		♂		♀		♂		♀		♂	♀
	Bot. 1.	Bot. 2.	Bot. 1.	Bot. 2.	Bot. 1.	Bot. 2.	Bot. 1.	Bot. 2.	Bot. 1.	Bot. 2.	Bot. 1.	Bot. 2.		
My 24	159 148													
25	200 238 264 224		133 161		144 138		121 112 116		158 121		104 126 98 115 113			
26	164 211 203		166 118 123		163 125 133 153 143 136 136		119 123 104		149 179 187 175 174 144 116		116 96 120 113 98 119 105 109 127	152 169 168 120 127 157 133	93 102 96	
27	133 184 136 132 205 129		106 105 71 82 80		177 169 155 245 138 198 142 153		89		172 169 183 178 153 153		111 122 98 116 112 111 113 80 108 129 124	161 161 144 131 146 155 149 146	93 113 106 113 112 112 113	
Je 7, 8	104 81 126 94 154 97 136	147 114 133 108 141 118 101 89	84 54 83 65 72 54	58 64 99 32 40 52 56 63 73	166 239 158 111 151 163 96 83	92 123 107 96 170 108 98 125 114 Full 134 126 61 94 95 114 195	49 57 57 51 77 46 47 88 87	34 76 140 62 45 77 54 46 52 41 34 31 48 104 87 37 32 36 100	102 150 116 128 91 109 72 81 127 92 121 103 150 111 141 92 123	97 72 54 85 135 118 65 41 35 60 66 105 83 109 85 92 80 48 57	66 33 54 32 53 65 30 47 48 81 68 55 55 85 37 91 87 57	43 75 85 81 51 30 41 35 60 66 68 50 55 81 37 91 87 43	94 115 140 96 139 140 128 109 89 104 87 85 150	28 49 88 42 83 37 70 52 44 56

Return selections from the low lines produced on the average slightly higher numbers than low selections in the same generations. Individual cases, however, do not seem to show any definite response. The few return selections made from the

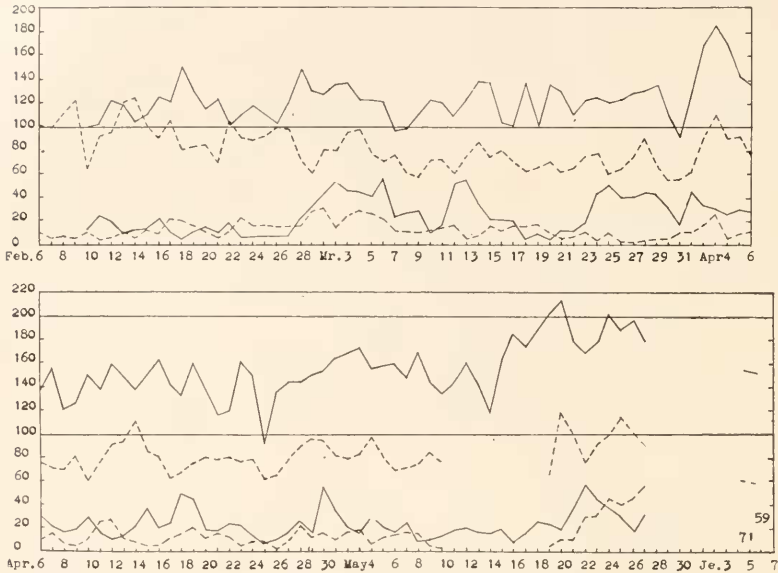


FIG. 8. Daily averages of high and low lines in Ba from February 6 to June 7, 1916. The high lines are represented by continuous lines and the low ones by broken lines. The two upper lines show the daily facet averages while the lower ones represent the actual numbers of flies from which those averages were obtained.

high lines were even less definite. Only one gave offspring that could be distinguished from those of the generation in which they hatched (u4f4-19).

The offspring from the crosses between low and high lines are intermediate and show a very slightly wider range than the low lines, but not nearly so wide a range as the high lines. There is no significant difference between the offspring from high males and low females and those from low males and high females. The apparent difference in the extremes is accounted for by the fact that the standard of all the lines changed during the period of hatching. The results of these matings are given in actual daily counts in Tables III. and IV., and are also shown in Figs. 5 and 7. A comparison of Tables III. and IV. with Fig. 8 shows

that the lower values in the later counts are entirely in agreement with the results obtained at this time in the high and low lines.

Such changes in facet numbers of all the lines had already been noticed in the vestigial-winged race and are clearly shown at several points in Fig. 8. The changes would appear more sudden and the agreement between the lines would be closer if it were not for the fact that the change appeared sooner in bottles that just began to hatch than in older bottles.

#### THE APPEARANCE OF HETEROZYGOUS FEMALES AND FULL-EYED MALES.

What developed to be the most puzzling phase of the entire set of experiments was the appearance of full-eyed males and heterozygous females in the stock bottles and in the selected lines during the period they were under observation. Eleven such individuals appeared at different times as shown in Table V.

TABLE V.

PEDIGREE OF FULL-EYED MALES AND HETEROZYGOUS FEMALES OBTAINED FROM BAR-EYED STOCK UNDER OBSERVATION.

Sex.	Date of Appearance.	Line.	Facets.	Fate.	Grade of Mates.	Bottle No.	Offspring.			
							♂		♀	
							Bar.	Full.	Bar.	Het.
♂	Nov. 15, 1915	VBa (Stock)	700?	Mated	43	VBax	10	2		76
♂	Nov. 29, 1915	VBa (Stock)	1200?	Died						
♂	Dec. 17, 1915	VBah15	1200?	Sterile						
♀	Jan. 16, 1916	VBah3f2	371	Mated	{ 109 116	VBah3f2-6	4	4	1	4
♂	Feb. 12, 1916	Bau2	1200?	Mated	{ 61 67	Bau2x	13			10
♀	Mar. 10, 1916	Bau2f2-3	601	Died						
♀	Mar. 28, 1916	Bau4f2-10	700?	Mated	149	Bau4f3x	27	17 <sup>1</sup>	20 <sup>1</sup>	18
♀	Apr. 2, 1916	Bau4f2-13	481	Mated	{ 238 204	Bau4f2-13x	3	3	2	4
♀	Apr. 28, 1916	Bau4f4-16	621	Mated	187	Bau4f5x	11	13	7	11
♀	June 6, 1916	Bau4f6-4	452	Killed						
♂	June 7, 1916	Baud8	800?	Killed						

<sup>1</sup> The full-eyed males and bar-eyed females in Bau4f3x were undoubtedly due to the appearance of flies from the second generation before the counts were made. The parents were mated April 3 and by an oversight the offspring were permitted to remain in the bottle until April 27.



The VBa stock in March revealed no such specimens, but the Ba stock in June contained several of each. In that case they were probably due to the appearance of a heterozygous female in the previous generation. One of the stock bottles received from Professor Zeleny contained a mixture of bar-eyed, full-eyed and heterozygous individuals. These may have been due to the appearance of a full-eyed male or a heterozygous female at some previous time.

The eyes of the males could not be distinguished from those of normal wild flies. The facet numbers indicated in the table are mere estimates obtained by counting a row of facets to the middle of the eye and using the number obtained as a radius for computing the number on the entire area. The eyes of wild flies treated in the same way gave similar results. The eye of a wild male which could be counted without turning was found to contain 700 facets.

The eyes of the heterozygous females had the characteristic appearance of the eyes of such females obtained from crosses between wild and bar-eyed flies and contained the corresponding facet numbers. The ordinary bar eye has the shape of a crescent with a notch near the middle of the concave side almost separating the two ends of the crescent. One end is usually considerably larger than the other and frequently contains the irregular facets mentioned before. The eye of the heterozygous female has the shape of the bar eye, but is much larger and does not contain the irregular facets. As shown in the table, the heterozygous females from the long-winged race had considerably larger eyes than those from the vestigial-winged race. This is explained by the fact that the average facet number in the bar eye of the long-winged race is much larger than that of the vestigial-winged race.

Wherever possible the full-eyed males and heterozygous females that appeared were mated, but, with the exception of the first case, no attempt was made to get a large number of offspring or to count the facets in the bar-eyed and heterozygous offspring. Two of the individuals died soon after hatching and the last two could not be mated because the experiments had to be brought to a close. Of those mated one proved to be

sterile and the other six produced offspring. In each case the specimens were mated with bar-eyed flies and with one exception the full-eyed males produced nothing but bar-eyed males and heterozygous females and the heterozygous females produced bar-eyed and full-eyed males and bar-eyed and heterozygous females. The one exception occurred in case of the male which appeared on March 28 and was mated to a bar-eyed female on April 3. The bottle was set aside and overlooked until April 27 when it was found to contain all four classes of flies as shown in the table. In that case the full-eyed males and bar-eyed females are undoubtedly due to the appearance of flies of the second generation before the count was made. Since the first generation consisted of bar-eyed males and heterozygous females the second generation would be expected to contain a mixture of all four classes.

The facets in the eyes of all the offspring obtained from the male appearing on November 15 were counted and the results are given under VBax in Table I. and Fig. 2. A large number of pairs from these offspring was mated, but all proved to be sterile. A mass mating made toward the end of the hatching period, however, produced 14 bar-eyed males with an average facet number of 114, 7 full-eyed males, 12 bar-eyed females with an average facet number of 91 and 5 heterozygous females. These were bred in a stock bottle for several generations to see if one or the other class would become dominant, but no obvious change in the ratios appeared.

#### DISCUSSION AND CONCLUSIONS.

The variability of the stock at the beginning of the selections made it appear probable that the facet number is affected by environmental conditions. For that reason the records during the experiments were kept more carefully than would otherwise have been the case. Counts were made and recorded separately at least every twelve hours. Each line or branch of a line was recorded separately, and in cases where parents were transferred and offspring obtained from both bottles a separate record was kept of each. Observations were also made on a possible correlation between body size and facet number.

It was impossible to find any noticeable effect of moisture, consistency, or age of food on the facet number. No significant difference was found in facet number between the first flies hatching from a bottle and the last ones except in cases where the food gradually dried up and the last flies were minute, *i. e.*, one half the size of normal flies or less. Such minute flies showed a tendency toward lower facet numbers. Accurate size measurements and facet counts on a large number of flies may possibly reveal a slight correlation between size of body and number of facets, but mere observation failed to detect it. It is also possible that a slight correlation exists but is concealed by other factors that have more influence.

No relation could be discovered between the age of the parent and the facet number of the offspring. In case of such a relation the latest offspring from two parents would be different from the first. No such difference could be detected.

Likewise it was impossible to find any definite correlation between the length of the developmental period of the larva and the facet number of the adult. No special experiments were undertaken with material in which the exact developmental period was ascertained, but, since in productive bottles the period of hatching is much longer than the period during which the eggs are laid, the first larvæ must as a rule have a shorter developmental period than the last, and any difference due to the length of the developmental period should become evident.

A glance at Fig. 8 shows that beyond the daily fluctuations due to the fact that the number of flies examined was too small to be representative, there are larger, parallel fluctuations of the two lines. Since the food during the Ba selections was fairly constant in character it can not be regarded as the cause of these fluctuations. The temperature of the room in which the flies were kept varied between rather wide limits. Unfortunately no thermograph was available for the room at that time. There is no correlation between the outside temperature and the facet number; but the variations in the room temperature were independent of those in the outside temperature. Since experiments to determine the effect of temperature on the facet number were undertaken by E. W. Seyster of this laboratory during the

latter part of this work and on account of the lack of time no special effort was made to determine the exact relation between temperature and facet number. The fact that the change usually occurred one to three days sooner in bottles that just began to hatch than in old ones can be explained by the assumption that the effect was produced at an early stage in the developmental period. Since the first flies hatching from a bottle must have a shorter developmental period than the later ones they would be the first to show the effect.

It does not seem possible, however, that temperature is the sole cause of somatic variations. In the VBa selections it was observed that the two lines throughout averaged higher than the stock. In spite of that fact an examination of the extremes shows that most of the high flies were eliminated from the low lines. The rank of the average flies must, then, have been raised. The same can be said of the low lines in Ba. Here the elimination of high flies is much more pronounced and still the mean remains very near that of the stock. It is possible that the crowded condition of the larvæ in the stock bottles reduced the facet number, but the real cause may be something very different.

The results of the selections in VBa indicate that there is in this race only a single hereditary factor involved in the modification of the facet number. In spite of the fact that the mean of the stock is much lower than that of either line we must assume that under the same conditions it would lie somewhere between them, in other words, if the lines could have been reared under the conditions of the stock, the low lines would have gone slightly downward due to the elimination of high numbers and the high lines would have gone slightly upward due to the elimination of low numbers. Practically pure lines were established in the first generation. This, however, may be merely apparent, the lack of further effect of selection being due to the interference of somatic factors.

The male with 34 facets, giving rise to the brood 16f1-6, must be regarded as a mutant. This conclusion is based on the great difference between this male and its brothers in 16, the definite relation between the male parent and the female offspring, and the extremely low grade of the resulting females.

In the Ba selections the results are very different and not so easily explained. Here also there must have been a downward shift as well as an upward shift. Disregarding the seventh generation for reasons already given, the following differences between the high and low lines are obtained: f<sub>1</sub>, 22; f<sub>2</sub>, 40; f<sub>3</sub>, 61; f<sub>4</sub>, 65; f<sub>5</sub>, 79; f<sub>6</sub>, 95. There is a gradual increase which becomes somewhat smaller as selection proceeds. If it were not for the interfering fluctuations the author's results would be much like those obtained by Zeleny and Mattoon. Even three additional generations of selection failed to produce pure lines.

In many respects the results obtained in these experiments resemble those obtained by MacDowell in the selection for extra bristles. The average of all the lines is raised or lowered but one can not predict within rather wide limits just what the offspring of any two parents are going to be like. This is clearly shown in Table II. and Fig. 6 in case of the matings dif1-2 and dif1-3. These matings were made with the same male but with females of 91 and 56 facets respectively. The average for the offspring from the higher female is considerably lower than that for the offspring of the lower female. In this case the unexpected results can not be explained on the basis that only a part of the character was observed. It is true that the counts were made only on one eye, but the difference between the two eyes falls within definite limits and is very small compared with the differences between the eyes of two flies. A comparable case would be the possibility of less than one extra bristle outside of the observed rows in the experiments of MacDowell. The large, parallel fluctuations of all the lines seem to indicate that there are environmental factors capable of almost doubling or cutting in half the facet numbers of flies of the same germinal constitution. The unexpected results must be regarded as being due to the fact that the hereditary factors are only a part of the total factor group controlling the facet number. When the environmental factors have been studied it may be possible to control them in such a way as to obtain uniform results. In that case more rigid selection will be possible.

The crosses made between the high and low lines show no evidence of sex-linkage. The offspring from both sets of matings

are very nearly alike and there is no essential difference in the ratio of males and females. This agrees with the results obtained by MacDowell in his work on extra bristles. In the present experiments sex-linkage might have been expected from the fact that the ratio between male and female averages did not remain constant from generation to generation. The factors for converting the female means to the male standard in the seven generations of upward selection are successively 1.91, 1.73, 1.53, 1.40, 1.36, 1.60, 1.57. Those for the downward selections are 1.57, 1.86, 1.43, 1.79, 1.51, 1.46 and 1.40. But it is impossible to relate the differences in the offspring to differences in the parents.

The appearance of full-eyed males and heterozygous females in the stock and selected lines may be explained in two ways. It may have been due to the carelessness of the author in preparing the food, feeding and transferring the flies, or it may have been due to reverse mutation in the race.

The precautions taken by the author were not such as to exclude absolutely the possibility of the entrance of an egg here and there. But in spite of that fact the evidence is almost irrefutably against the theory of contamination. Perhaps it is well to state here in detail the author's methods. The precautions in regard to the preparation of food have already been given. It is sufficient to add that no larvæ ever appeared in the food jar. In all of these experiments the food was handled by means of an all-metal scalpel. This was used chiefly because it could be easily cleaned and could be kept absolutely clean. During the first two generations of VBa selections the author depended upon the fact that *Drosophila* does not as a rule lay eggs on a clean, dry, metal surface and merely kept the scalpel clean but did not heat it before using. In all succeeding work it was heated in an alcohol flame just before being used. All vials and bottles that had been used were boiled and rinsed in tap water and were then kept inverted on the table until they were again used. In the VBa selections no filter paper was used with the food. In the Ba selections the clean filter paper was kept in a table drawer and was removed only at the time of using. Fresh cotton for the plugs was also kept in table drawers.



If old plugs were used over again they were either sterilized and kept in closed fruit jars or they were kept in closed jars for at least a week before using. Only plugs that had not been contaminated with food were used again.

But the evidence against the theory of contamination has little to do with the precautions taken in handling the material. In the first place all flies that appeared in the vestigial-winged lines had vestigial wings and all flies that appeared in the long-winged lines had long wings. The chief reason for choosing the vestigial-winged race for the selections was the fact that the second recessive could be used as a check in case of contamination. In all of the experiments no long-winged fly appeared in the vestigial-winged lines and no vestigial-winged form appeared in the long-winged lines. If the full-eyed and heterozygous flies had been due to contamination then the other characters should also have appeared, especially since for a long time the breeding vials of the two races were intermingled and treated as one lot. In the second place the author was handling no full-eyed, vestigial-winged flies at the time the first male appeared. It is true that Professor Zeleny had his stock of such flies in the same room at the time, but they were kept on a table about twenty feet from the one used for this work and the chances that a vestigial-winged fly will travel that distance are not very great. Finally, if the flies were due to contamination, full-eyed females as well as full-eyed males should have appeared; indeed, full-eyed females should have been more frequent than heterozygous females, but all females were heterozygous.

It is interesting to note that in all cases except one where full-eyed or heterozygous flies appeared in the selected lines it was in the high lines. The one exception was the heterozygous female in  $h3f2$ , a reverse selection from a low line.

More significant, however, is the fact that the females were always heterozygous. That means that if the change took place after fertilization only one chromosome in case of the females was affected. The males, of course, have only one chromosome bearing the factor. If the change occurred before fertilization, then it is probable that it appeared in the female germ cells. Had it taken place also in the male germ cells then



heterozygous females should have appeared more frequently than full-eyed males and full-eyed females might in rare instances have appeared. If the change occurred indiscriminately in male or female then the proportion of heterozygous females to full-eyed males should be 3 to 1, but the ratio obtained is 5 to 6. The numbers obtained, however, are not large enough to be conclusive.

The fact of reverse mutation is very difficult to explain. It is hard to see how the germplasm can lose a factor and still potentially retain it and have it reappear later. So far as the present data go it is possible to explain the case under consideration in two different ways.

If the normal wild fly carries a limiting factor with respect to the facet number then it is possible by partial non-disjunction for the factor to pass from one chromosome of a pair to the other, giving one chromosome without a limiting factor and the other with a double limiting factor. The bar-eyed race of *Drosophila* may be derived from such a chromosome with two limiting factors or factor groups, the mate of the chromosome having been lost in the maturation of the egg. If then in the bar-eyed race a second non-disjunction again separates the two factors the result should be one chromosome with triple factors and one with a single factor, the latter giving rise to a full-eyed male or a heterozygous female. If the former passes into the egg it should give rise to a further reduction in the facet number, but it is possible that a fly with such a chromosome does not live. It is possible also that the male with 34 facets contained a chromosome with a triple reducing factor.

A simpler explanation is that of a reversible chemical change between two compounds one of which is more stable than the other. If the compound that forms the basis for the bar eye is the less stable then reversions are to be expected under certain conditions. But the fact that the change takes place only in the female is a strong argument in favor of the theory of partial non-disjunction.

#### SUMMARY.

Selection was carried on in the vestigial-winged, bar-eyed stock for three generations and in the long-winged, bar-eyed

stock for seven generations. The facets in the eyes of 9,000 flies were counted.

Selection in the vestigial-winged stock had to be discontinued on account of sterility and low production in the single lines.

The sterility and low production were not due to inbreeding. Sterility was not due to inability on the part of the females to lay eggs, nor does it appear to have been the final stage in the reduction of the number of offspring.

Selection in the vestigial-winged stock was effective for one generation but failed to produce further effects in the second and third generations. Return selections from the low lines were not effective.

A mutation appeared in a male in line 16. It was a reducing factor and was sex-linked. The complete sterility of the offspring of this male prevented further study of the character.

Selection in the long-winged stock was effective for six generations. In the downward selected lines most of the high flies were eliminated in the first three generations and no further effect of selection was noted. Return selections from the sixth generation, however, were still effective. The mean of the upward selected lines continued to increase at approximately uniform rate for six generations. The seventh generation must be disregarded because it was not an upward selection. The range in the facet number was not increased by selection. The results of these experiments indicate that the hereditary differences in this race of *Drosophila* are due to a large number of small factors.

Crosses made in the sixth generation between low and high lines indicate that the hereditary factors are not sex-linked.

In both the long-winged and the vestigial-winged lines the mean facet number was highly variable. This variability appeared to be due chiefly to changes in the temperature of the room, but may have been due also to other causes. The mean facet number of flies reared in vials as single lines was slightly higher than that of stock flies reared in bottles.

The mean facet number of the vestigial-winged flies was somewhat lower than that of the long-winged flies.

Six full-eyed males and five heterozygous females appeared in the stock bottles and the selected lines during these experiments. They must be regarded as reverse mutations.

The reverse mutation can be explained on the theory of a reversible chemical reaction between two compounds one of which is more stable than the other. The present facts, however, favor an explanation on the basis of partial non-disjunction.

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